



Impaired right ventricular-pulmonary vascular function in myeloproliferative neoplasms

Emir C. Roach, MD,^a Margaret M. Park, BS,^b W.H. Wilson Tang, MD,^b
James D. Thomas, MD,^b Kewal Asosingh, MD,^a Matt Kalaycio, MD,^c
Serpil C. Erzurum, MD,^{a,d} and Samar Farha, MD^{a,d}

From the ^aDepartment of Pathobiology, Lerner Research Institute; the ^bHeart & Vascular Institute; the ^cTaussig Cancer Institute; and the ^dRespiratory Institute, Cleveland Clinic, Cleveland, Ohio.

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BACKGROUND: Increased bone marrow hemangioblast numbers, alterations in erythroid/myeloid lineages, increased reticulin, and greater circulating bone marrow progenitor cells are present in patients with pulmonary arterial hypertension (PAH). The data suggest that myeloid progenitors contribute to the pathogenesis of PAH, but there are little data on the prevalence of pulmonary vascular disease among the different forms of myeloid diseases. We hypothesized that there would be a higher prevalence of pulmonary vascular disease in myeloproliferative neoplasms that have high circulating progenitor cells, such as myelofibrosis and chronic myelogenous leukemia (CML), compared with those with low circulating progenitors, such as in aplastic anemia.

METHODS: Patients with myelofibrosis, CML, and aplastic anemia who underwent echocardiographic evaluation of cardiac function in preparation for bone marrow transplantation at the Cleveland Clinic between 1997 and 2012 were identified and their electronic medical records were queried for demographic data, blood cell counts, and pulmonary function tests. All echocardiograms were uniformly analyzed in a blinded fashion by an advanced sonographer and cardiologist for measures of right and left ventricular function and estimation of pulmonary vascular disease.

RESULTS: Gender and race distribution among disease groups was similar. Patients with myelofibrosis ($n = 19$) and aplastic anemia ($n = 30$) had increased right ventricle (RV) wall thickness compared with CML ($n = 82$) patients (aplastic anemia, 0.7 ± 0.1 ; CML, 0.5 ± 0.1 ; and myelofibrosis, 0.7 ± 0.1 ; $p = 0.02$). Patients with myelofibrosis had higher levels of estimated RV systolic pressure compared with the other groups (aplastic anemia, 29.9 ± 1.5 ; CML, 26.2 ± 1.1 ; and myelofibrosis, 36.7 ± 3.7 mm Hg; $p < 0.01$).

CONCLUSIONS: The findings suggest an important role for myeloid progenitors in the maintenance of pulmonary-vascular health, in which abnormal myeloproliferative progenitors are associated with RV pathology.

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Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary vascular resistance and elevated right ventricular pressure leading to permanent

changes in the pulmonary vasculature, right ventricular failure, and death. The association between myeloproliferative neoplasms and PAH has been suggested by case reports and small series, but the prevalence of pulmonary vascular abnormalities in myeloid diseases is unknown.^{1,2} Patients with myeloproliferative diseases are at risk of developing PAH.³ Likewise, patients with PAH are prone to develop overt myelofibrosis (MF) and/or thrombocytopenia.

Reprint requests: Samar Farha, MD, Cleveland Clinic, 9500 Euclid Ave, NC22, Cleveland, OH 44195. Telephone: +216 445 6624. Fax: +216 636 0104.

E-mail address: farhas@ccf.org

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Popat et al⁴ found MF uniformly in the specimens of all PAH patients who underwent bone marrow biopsy. More recently, we have shown that reticulin fibrosis is present in patients with idiopathic and familial PAH and even in non-affected family members of PAH patients with blood counts within normal reference ranges.⁵

Myeloproliferative neoplasms are characterized by a common stem cell-derived clonal proliferation but are phenotypically diverse due to differences in genetic rearrangements or mutations. They usually exhibit terminal myeloid cell expansion in the peripheral blood.⁶ An increased number of circulating hematopoietic progenitors has been described in myeloproliferative neoplasms, with the highest numbers observed in MF.^{7–9} Similar to myeloproliferative neoplasms, bone marrow-derived proangiogenic precursors are increased in the circulation of PAH patients compared with healthy controls.¹⁰ In addition, there are increased proliferation of hemangioblasts in the bone marrow, alterations in erythroid/myeloid lineages, and increased reticulin fibrosis in the bone marrow of PAH patients. The relationship of the number of circulating progenitor cells to the severity of PAH suggests a possible role for these cells in fueling the angioproliferative vascular remodeling in PAH.⁵ Moreover, transplantation of bone marrow CD133+ stem cells from PAH patients into immunodeficient mice recapitulates key features of the diseases, including endothelial cell injury, in situ thrombi, and right ventricular hypertrophy.¹¹ All of this supports a possible causal link between the myeloid abnormalities and PAH. In fact, there are case reports identifying PAH resolution with treatment of the myeloproliferative disease.^{3,12,13} Conversely, PAH was reported to be a complication of stem cell transplantation in 40 patients in the literature.¹⁴

To further delineate the role of bone marrow-derived hematopoietic stem cells in the development of PAH, patients with myeloproliferative neoplasms were identified and compared with patients with aplastic anemia (AA), a hematologic disease reflecting a deficiency of hematopoietic stem cells resulting in pancytopenia and bone marrow aplasia. We hypothesized that right ventricular and pulmonary vascular abnormalities would be associated with the elevated circulating progenitor cells in myeloproliferative processes compared with myeloprogenitor-deficient AA.

Methods

The Cleveland Clinic Institutional Review Board approved this study.

Study population and data collection

The study included patients who underwent allogeneic stem cell transplantation for AA, MF, or chronic myelogenous leukemia (CML) from 1997 to 2012 at the Taussig Cancer Institute, Cleveland Clinic. This population was selected because patients who are considered for transplant undergo a screening echocardiogram at the Cleveland Clinic. Data were collected from the bone marrow transplant registry and the medical records.

We identified 131 patients who were eligible for the study. We reviewed demographics, blood counts and chemistry values,

pulmonary function tests, and echocardiograms. Two-dimensional (2D) echocardiograms performed from 1 to 3 months before transplant were analyzed by an advanced sonographer and reviewed by an experienced cardiologist blinded to the patient's diagnosis.

Statistical analysis

All analyses were performed using JMP Pro 9.0 software (SAS Institute Inc, Cary, NC). Descriptive measures for quantitative variables consist of means \pm the standard error of the mean. Comparisons of disease groups were performed using analysis of variance. When analysis of variance was significant, the Tukey honest significant difference test was performed for pairwise comparison for post hoc analysis.

Results

The baseline characteristics of the 131 study patients, subgrouped by each disease, are reported in [Table 1](#). MF patients were older than CML and AA patients ($p < 0.001$). The gender and race distribution among the disease groups was not significantly different ($p > 0.1$). As expected, blood cell counts were significantly different among groups ([Table 1](#)). Patients with AA had lower cell counts than the other groups.

Lung functions

Pulmonary function testing done before transplantation was compared among disease groups ([Table 1](#)). Forced vital capacity (% FVC) was significantly lower in the MF group ($p = 0.03$). There was no difference in force expiratory volume in 1 second (FEV₁) and FEV₁/FVC (all $p > 0.05$). Diffusion capacity of the lung for carbon monoxide was significantly higher in the CML and MF groups than in the AA group, but the differences were not significant when adjusted for hemoglobin ([Table 1](#)).

Echocardiographic measures of cardiopulmonary disease

Echocardiograms were available for repeat analyses in 131 individuals 1 to 3 months before bone marrow transplantation. Left-sided function was not different among the groups (left ventricular ejection fraction: AA, 57.1% \pm 0.7%; CML, 58.3% \pm 0.6%; and MF, 59.6% \pm 0.8%; $p = 0.2$; [Table 2](#)). However, left atrial (LA) systolic dimension was significantly higher in MF patients (AA, 3.3 \pm 0.2; CML, 3.6 \pm 0.1; and MF, 4.2 \pm 0.2 cm; $p < 0.01$). LA biplane volume was measured but was only available in 10 patients (AA, 82 \pm 34; CML, 54 \pm 4; and MF, 84 cm²; $p = 0.6$).

The groups did not differ significantly at baseline right atrial (RA) pressure (AA, 14.7 \pm 1.0; CML, 17.1 \pm 0.8; and MF, 18.9 \pm 1.5 mm Hg; $p = 0.8$) and tricuspid annular plane systolic excursion (AA, 2.0 \pm 0.1; CML, 2.4 \pm 0.1; and MF, 2.3 \pm 0.2 cm; $p = 0.7$). However, compared with the other 2 groups, MF patients had significantly higher

Table 1 Baseline Characteristics of Disease Groups

| Variables ^a | CML (n = 82) | Myelofibrosis (n = 19) | Aplastic anemia (n = 30) | p-value |
|--|-----------------|---------------------------|-----------------------------|---------|
| Age, years | 42 ± 1 | 56 ± 1 | 35 ± 3 | <0.001 |
| Race | | | | |
| Caucasian | 68 | 19 | 27 | |
| African-American | 9 | 0 | 1 | |
| Asian | 1 | 0 | 0 | 0.5 |
| Hispanic | 2 | 0 | 0 | |
| Other | 2 | 0 | 2 | |
| Gender | | | | 0.7 |
| Male | 41 | 8 | 16 | |
| Female | 41 | 11 | 14 | |
| Hemoglobin, g/dl | 11.8 ± 0.2 | 9.3 ± 0.4 | 9.1 ± 0.3 | <0.001 |
| White blood cell count, ×10 ³ /μl | 14.4 ± 2.6 | 21.1 ± 5.3 | 0.8 ± 0.2 | 0.005 |
| Red blood cell count, ×10 ⁶ /μl | 3.8 ± 0.1 | 3.3 ± 0.2 | 3.0 ± 0.1 | <0.001 |
| Hematocrit, % | 35.6 ± 0.6 | 29.1 ± 1.3 | 25.7 ± 0.9 | <0.001 |
| MCV, fl | 94.8 ± 1.0 | 88.6 ± 1.8 | 86.2 ± 1.5 | <0.001 |
| MCH, pg/cell | 31.2 ± 0.4 | 28.3 ± 0.8 | 29.2 ± 1.2 | 0.02 |
| MCHC, g/dL | 33.2 ± 0.1 | 31.9 ± 0.4 | 35.3 ± 0.1 | <0.001 |
| Red cell distribution width, % | 16.7 ± 0.4 | 19.8 ± 0.7 | 16.3 ± 0.7 | <0.001 |
| Platelet count, × 10 ³ /μl | 269 ± 28 | 264 ± 65 | 18 ± 2 | <0.001 |
| FEV ₁ , % | 91 ± 2 | 87 ± 3 | 94 ± 3 | 0.2 |
| FVC, % | 97 ± 2 | 89 ± 3 | 98 ± 3 | 0.03 |
| FEV ₁ /FVC | 78 ± 1 | 78 ± 1 | 79 ± 1 | 0.7 |
| D _{LCO} , % | 86 ± 2 | 73 ± 3 | 64 ± 4 | <0.001 |
| D _{LCOc} % | 91 ± 3 | 84 ± 4 | 82 ± 4 | 0.2 |

CML, chronic myelogenous leukemia; D_{LCO}, diffusion capacity of the lung for carbon monoxide; D_{LCOc}, diffusion capacity of the lung for carbon monoxide corrected for hemoglobin concentration; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

^aContinuous data are shown as mean ± standard error of the mean and categorical data as number.

right ventricular (RV) systolic pressure (RVSP; AA, 29.9 ± 1.5; CML, 26.2 ± 1.1; and MF, 36.7 ± 3.7 mm Hg; *p* < 0.01) and RA area average (AA, 4.0 ± 1.0; CML, 3.3 ± 0.8; and MF, 5.3 ± 1.6 cm²; *p* = 0.04). RV fractional shortening tended to be higher in MF patients (AA, 32.7 ± 2.2; CML, 34.2 ± 2.3, and MF, 41.1 ± 3.2; *p* = 0.08; [Table 2](#)). The RV was significantly thicker in MF and AA patients compared with CML patients (AA, 0.7 ± 0.1; CML, 0.5 ± 0.1, and MF, 0.7 ± 0.1 cm; *p* = 0.02; [Figure 1](#)). In addition,

interventricular septal diastolic thickness was different among the groups (AA, 0.98 ± 0.04; CML, 1.04 ± 0.02; and MF, 1.18 ± 0.06 cm; *p* = 0.005).

Discussion

The association of PAH with MF and other myeloproliferative disorders has been reported in case series. However, the exact prevalence remains unknown and is likely to be

Table 2 Differences in Echocardiographic Parameters Among Myeloid Diseases

| Variables ^a | CML | Myelofibrosis | Aplastic anemia | p-value |
|---|-------------|---------------|-----------------|---------|
| Left atrial systolic dimension, cm | 3.6 ± 0.1 | 4.2 ± 0.2 | 3.3 ± 0.2 | <0.01 |
| Left ventricular | | | | |
| Ejection fraction, % | 58.3 ± 0.6 | 59.6 ± 0.8 | 57.1 ± 0.7 | 0.2 |
| End diastolic dimension, cm | 4.8 ± 0.1 | 4.8 ± 0.1 | 4.7 ± 0.1 | 0.8 |
| Interventricular diastolic septal thickness, cm | 1.04 ± 0.02 | 1.18 ± 0.06 | 0.98 ± 0.04 | 0.005 |
| Right ventricular | | | | |
| Systolic pressure, mm Hg | 26.2 ± 1.1 | 36.7 ± 3.7 | 29.9 ± 1.5 | <0.01 |
| Thickness, cm | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.02 |
| Fractional shortening, % | 34.2 ± 2.3 | 41.1 ± 3.2 | 32.7 ± 2.2 | 0.08 |
| Tricuspid annular plane systolic excursion, cm | 2.4 ± 0.1 | 2.3 ± 0.2 | 2.0 ± 0.1 | 0.7 |
| Right atrial | | | | |
| Pressure, mm Hg | 17.1 ± 0.8 | 18.9 ± 1.5 | 14.7 ± 1.0 | 0.8 |
| Area average, cm ² | 3.3 ± 0.8 | 5.3 ± 1.6 | 4.0 ± 1.0 | 0.04 |

CML, chronic myelogenous leukemia.

^aData are shown as mean ± standard error of the mean.

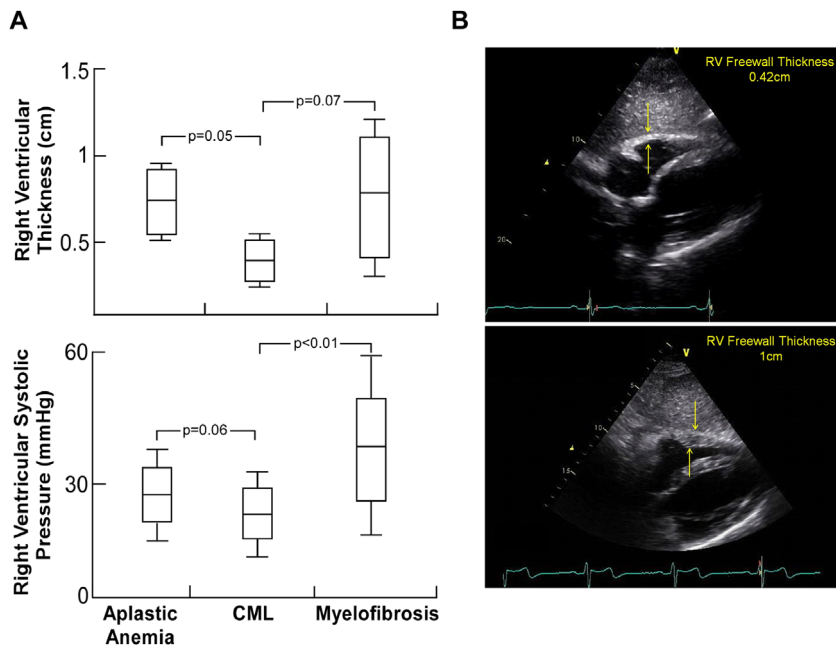


Figure 1 (A) The right ventricle (RV) was thicker in patients with aplastic anemia and myelofibrosis than in patients with chronic myelogenous leukemia (CML; $p = 0.02$ by analysis of variance). RV systolic pressure (RVSP) was highest in patients with MF, with average levels that would qualify as pulmonary hypertension (mean RVSP, 36.7). Aplastic anemia patients tended to have higher RVSP than patients with CML, but levels were generally lower than those that would qualify as pulmonary hypertension ($p < 0.01$ by analysis of variance). The p -values for comparison of mean between groups was obtained using the Tukey honest significant differences test. The horizontal line in the middle of each box indicates the median; the top and bottom borders of the box mark the 75th and 25th percentiles, respectively; and the whiskers mark the 90th and 10th percentiles. (B) RV free wall thickness measured by echocardiography. Upper and lower panels show normal and pathologic thickening of the RV wall, respectively.

underestimated. This study represents a large evaluation of cardiopulmonary function of patients with myeloid disease. Similar to prior reports,^{3,15} significant right heart changes are found in patients with MF, including elevated RVSP and RV thickness. Unexpectedly, AA patients also had thickened RVs, although RVSP was not elevated. Although the data from the AA patients are limited, this report describes RV thickness abnormality in this population. The findings suggest that myeloid progenitors are important to maintain RV function; that is, too few or too many circulating myeloid progenitors appear to be associated with alterations in the right heart size or structure. Just as important, the LV was not affected across any of the myeloid diseases, indicating right heart and pulmonary vascular localized effects of myeloid elements.

MF patients have higher numbers of circulating CD34+/CD133+ bone marrow progenitor cells than patients with other myeloid diseases.¹⁶ Levels of circulating CD34+/CD133+ bone marrow-derived proangiogenic precursors are similarly high in PAH patients.¹⁰ Moreover, non-affected members had reticulin fibrosis and increased myeloid progenitor cells similar to affected members, suggesting that myeloproliferative disease precedes the development of PAH.⁵ Interestingly, mice transplanted with CD34+/CD133+ bone marrow-derived progenitor cells from PAH patients develop pulmonary vascular endothelial injury, angioproliferative remodeling, and RV hypertrophy and failure.⁹ Here, cardiac structure and function were evaluated in patients with myeloid diseases, which are known to have high, or low, levels of circulating

progenitors. Although CML patients have high levels of circulating bone marrow progenitors, these individuals did not have increased RV thickness or elevated RVSP. Rather patients with AA, who have the lowest progenitors, had increased RV thickness, similar to MF patients, albeit with no elevation of RVSP. On the basis of these data, it is interesting to speculate that myeloid effects on the RV may be independent of pulmonary vascular effects.

Bone marrow-derived progenitor cells have been reported to repair or regenerate somatic cells in homeostasis or injury settings.¹⁷ An autopsy study of gender-discordant bone marrow transplantation found a significant fraction of the transplant recipient's cardiomyocytes was derived from the donor bone marrow-progenitor cells.¹⁸ Important to the current findings, a prior report showed that allogeneic bone marrow transplantation improved cardiac function in an AA patient.¹⁹ These reports, together with the finding of RV changes in AA in this study, indicate that hematopoietic progenitor cells are important to maintain RV homeostasis. Although others and we focus on the circulating progenitor cells, it is also possible that paracrine factors produced by the resident bone marrow cells may be supportive of right heart and pulmonary vascular health.

Interestingly, we compared post-transplantation outcomes in the different disease groups and found that ~75% of patients with AA were still alive, whereas ~65% of patients with MF had died at the time of data collection (data not shown). Outcomes did not differ based on the RVSP or RV thickness; however, as expected, there was a difference in outcomes based on the patient's age. It is hard

to draw conclusions without accounting for the different confounders for death related to factors including age and post-transplantation complications. Further studies are needed to assess differences in outcomes among the different disease groups specifically in relation to RV function.

One shortcoming of this study is that the evaluation of right heart pressures was not confirmed by right heart catheterization. This was not possible due to the retrospective nature of our study. In addition to possibly overestimating the rate of pulmonary hypertension in this population, secondary causes of PAH cannot be excluded. For example, portal hypertension can be associated with MF, high cardiac output state, chemotherapeutic agents, or extramedullary hematopoiesis, and all of these are known to be associated with pulmonary hypertension.²⁰ Whether these complications of myeloid diseases were present in the patients is unknown.

In conclusion, this study confirms the strong association between MF and PAH and reveals a new association between myeloid disease and RV abnormalities that is independent of changes in RVSP. The findings add to the growing evidence that myeloid progenitors are important in the maintenance of RV and pulmonary vascular homeostasis.

Disclosure statement

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